UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	**:**	**April 27, 2001**
LISZIEWICZ, et al.	**:**	**Atty Docket No. RGT 9771**
Serial No. 09/153,198	**:**	**Group 1633**
Filed: 15 September 1998	**:**	**Examiner: Wilson**

For: Method of Delivering Genes into Antigen Presenting Cells of the Skin

Commissioner for Patents
And Trademarks
Washington, D.C. 20231

DECLARATION

1. I, Dr. Julianna Lisziewicz, am co-director and founder of the Research Institute for Genetic and Human Therapy (RIGHT), Gaithersburg, MD and Pavia, Italy (1995-present), as well as Adjunct Associate Professor of Microbiology & Immunology at Georgetown University in Washington, D.C. (1996-present). I received a Master of Science in Chemistry in 1980 from the Technical University, Budapest, Hungary, and a Master of Science in Biochemistry from the same institution in 1982. I received my Ph.D in Molecular Biology from the Max Planck Institute for Experimental Medicine, Gottingen, Germany in 1986, and served as a Postdoctoral Research Associate at the same institution from 1986-1988. I was a Guest Researcher at the National Institutes of Health (NIH) in the Laboratory of Molecular Virology from 1988-1989 and in the Laboratory of Tumor Cell Biology in 1989-1990. I was head of the Antiviral Unit and Visiting Scientist at the same institution in the Laboratory of Tumor Cell Biology from 1990-1995. I have been involved in AIDS and cancer research since about 1988. I am one of the inventors named in the referenced application.

2. The following experiment was done under my supervision and control. It compares various modes of treatment for two groups of macaques with AIDS: those treated with continuous, Highly Active Antiretroviral Therapy ("HAART"), and those treated with the same HAART on an intermittent (three-weeks-on, three-weeks-off") schedule ("STI-HAART"). The survivors, all from the STI-HAART group, were subsequently treated according to the teachings of the present invention. In brief, the present invention induced an immune response capable of controlling HIV replication in monkeys *with AIDS.*

Background: Macaques with SIV_{251} : A model for HIV infection

1. We have relied on the infection of rhesus macaques by Simian Immunodeficiency Virus ($SIVmac_{251}$) as an animal model for HIV infection in humans because of the similarities to HIV infection in humans. Mucosal inoculation of macaques with $SIVmac_{251}$ reproducibly results in an infection characterized by peak plasma viremia within three weeks post infection, followed by a plateau which can persist for several months. Eventually, most animals will progress toward an acquired immune deficiency syndrome, characterized by high viral load and low CD4 counts. Occasionally, a small percentage of infected animals will manage to control virus replication spontaneously and exhibit very low levels of plasma viremia, similar to human long-term non-progressors [ref: 1,2].

2. Further, the animal model shows close parallels to the human disease with respect to the response to drug treatment, including responses to continuous treatment, treatment interruptions, drug toxicities, and drug interactions. For example, results from this study closely resembled the course of HIV infection in humans. Untreated animals developed chronic infections leading to a progressive increase of viremia and decrease of CD4 percentage. As with HAART treated patients, the animals treated continuously with the combination of PMPA, ddI and HU promptly controlled viral replication. Viremia remained undetectable and animals had normal CD4 counts as long as the drugs were administered. Similar to HAART

treated patients, viremia quickly rebounded and CD4 percentage rapidly decreased after antiretroviral therapy was interrupted.

3. The development of toxicity in this non-human primate model also mimicked the side effects of antiretroviral drugs in HIV infected patients. The study drugs induced severe iatrogenic diabetes, acute hepatitis and pancreatitis in the animals treated with continuous HAART. PMPA has been reported to be well tolerated in rhesus macaques [ref: 3,4] but, elevated alkaline phosphatase has been shown to occur in approximately 67% of PMPA-treated infants [ref: 6]. ddI is known to generate both hepatic and pancreatic toxicity, with relatively low frequency [Ref: 7-10]. Fulminant hepatitis and lactic acidosis have been also described in some HIV infected patients treated with ddI [ref: 11,12]. Like the patients, these animals had normal liver function before the fatal episode and ALT, AST and amylase values increased after therapy interruption. One case of diabetic syndrome has been reported following the treatment with ddI [ref: 13]. Pancreatitis has been recently reported in patients treated with ddI, stavudine with and without HU (ACTG 5025) [ref:14]. In this study ddI has been used QD at the dosage of 400 mg. In contrast, four randomized controlled studies using the 200 mg two times a day (BID) dose of ddI with HU did not report any case of pancreatitis [ref: 5,15-18]. Since the pancreatic toxicity of ddI is dose dependent, it is possible that, in rare cases, the increased peak level associated with the once daily dose of ddI could be partly responsible for this side effect. Nevertheless, the possibility exists that the same mechanism that induces synergy between HU with nucleoside or nucleotide analogues might also increase the toxicity of these drugs. All these results demonstrate that the course of disease and response to drug treatment in the animal model resembles that of the human disease.

4. It is accepted by researchers in the field that antiretrovial drug treatment of SIV_{251} infection in macaques produces results similar to those found in humans. See "Control of SIV Rebound Through Structured Treatment Interruptions during early infection" Lori, et al., Science 24 Nov 2000, v. 290, pp. 1591-1593, paragraph bridging col. 2 and 3 (copy enclosed) [Ref: 19]. That paper also describes a test for CTL response that correlates with the

ability to control HIV replication in the absence of drug treatment. See discussion of Fig. 3, starting with last paragraph on page 1592.

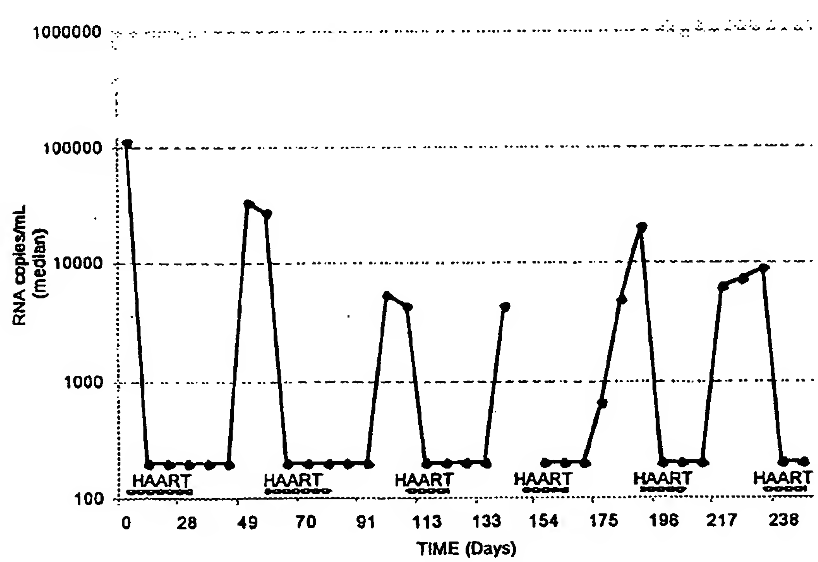
5. One principle underlying the referenced paper is that HAART has been shown to have therapeutic efficacy by inhibiting viral load and also improving the time of survival in both humans and monkeys. The paper shows that fixed-scheduled STI-HAART can induce immune control during drug treatment interruptions in SIV_{251}-infected monkeys treated early after infection, that Virus-specific T cell immunity (CD8VIR, a test that was developed based on studies of human long term non-progressors) was induced by STI-HAART but not by continuous HAART, and that CD8VIR was associated with the control of virus rebound during treatment interruptions. Similar data has been obtained in HIV-1 infected patients treated early after infection [ref: 20,21]. These findings suggested, among other things, that virus-specific T cell mediated immune responses have a therapeutic benefit, that can be detected by both analytical methods and by the reduction or absence of viral load rebound after HAART treatment interruption.

<u>Efficacy of Transcutaneous Immunization Treatment in SIV-infected Macaques with AIDS</u>

1. A cohort of ten SIV_{251} infected rhesus monkeys showing signs of AIDS was enrolled in a randomized study initially designed to compare continuous HAART with STI-HAART. Three animals died before the study was approved, demonstrating that the cohort was in the final stage of AIDS. Unfortunately, similar animals were not available to increase the size of the cohort. Consequently, 3 animals were randomized into the HAART and 4 animals in the STI-HAART arms.

2. Our animal cohort originated from a larger cohort of animals infected at the same time. Seven animals from this cohort died before initiation of treatment (439 days post infection). Animals treated with HAART survived an extra six months from the start of treatment. One animal in the STI-HAART group died at nine months from the start of treatment, that is, three months after the last animal in the HAART group died. The other three animals in the STI-HAART group were alive a year from the start of treatment. This survival study suggests that potent antiretroviral therapy, both continuous HAART and STI-HAART, can increase the time of survival of animals with AIDS. These results are similar to those found in AIDS patients treated with HAART. However, despite the successful HAART treatment, all animals treated continuously with potent antiretroviral therapy died within six months. The cause of death was either drug-related toxicity or AIDS, similarly that occurs in AIDS patients. In contrast, all the animals in the STI-HAART arm survived longer than six months.

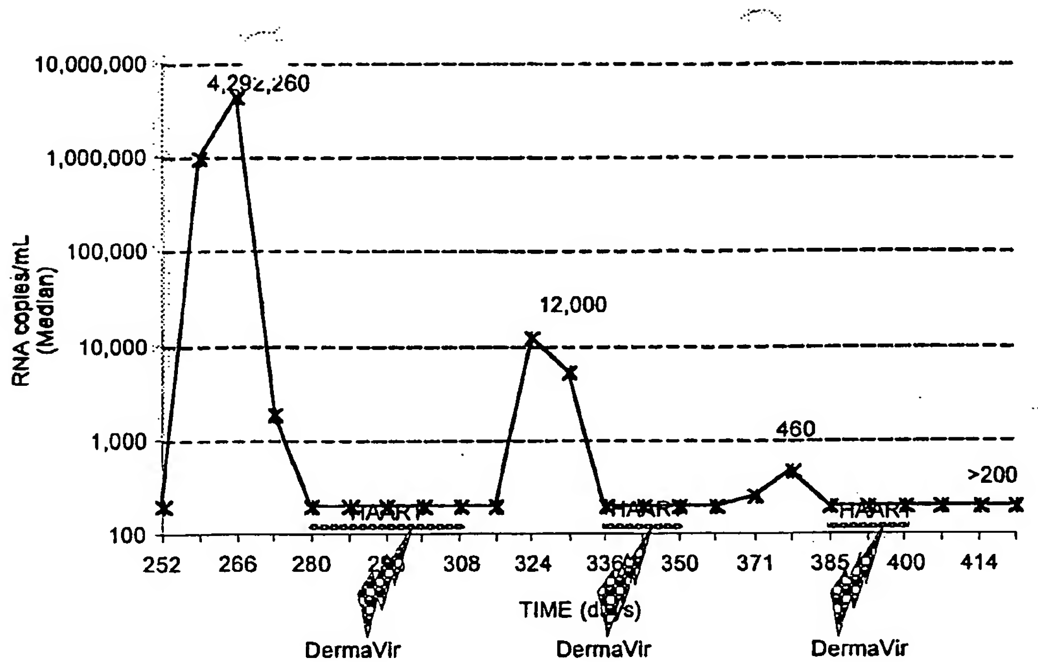
3. The STI-HAART treated animals with AIDS did not exhibit enhanced control of virus rebound during the treatment interruptions, a decreased rate of viral rebound, or improved control of viral replication during interruption of therapy (see figure). SIV-specific T cell immunity was not reconstituted during the



therapy. These results are consistent with those found in patients: During chronic HIV infection STI-HAART mobilized HIV-specific T cells only transiently, and the patients failed to control viral rebounds during treatment interruptions. These results indicated that, in both humans and monkeys with chronic HIV infection, autologous virus cannot be used to reconstitute HIV-specific T cell immunity sufficiently to control viral load.

4. At that point it became evident that these 3 animals might never control virus replication during treatment interruptions, and some signs of deterioration began to appear. In particular, the median viral load during the treatment interruption phase increased to 4,292,260 copies/mL, and the last HAART cycle failed to suppress viral load below undetectable levels in one animal. We decided to treat these 3 animals with a novel immune therapy, called DermaVir. DermaVir is a product name for an embodiment of the present invention, a complex of PEI-mannose and plasmid DNA encoding an integrase-defective SHIV (Simian-Human Immunodeficiency Virus) in sugar-water solution.

5. The animals continued the fixed-scheduled STI-HAART protocol, and DermaVir treatment was initiated in combination with HAART during the treatment periods. A figure relating the timeline, treatment schedule, and viral load is shown here. According to the schedule of the STI-HAART protocol, therapy was interrupted for three weeks.



6. During this first therapy interruption after the DermaVir treatment, the median viral rebound decreased from 4,292,260 to 12,000 copies/mL. The viral load in all the animals was 2-3 logs less than during the previous treatment interruption. Unexpectedly, two animals spontaneously controlled viral rebound even before therapy was re-initiated.

7. Three weeks of HAART in combination with DermaVir followed this three-week therapy interruption. The one animal that had not been able to control virus replication during the previous therapy cycle reached an undetectable viral load and the other two animals successfully controlled virus replication. This treatment period was followed by a three-week therapy interruption period.

8. During the second interruption period, the virus rebounded, but the median viral rebound decreased from 12,000 to 460 copies/mL. The one animal that was unable to control virus replication previously rebounded to 3,980 copies/mL. The other two animals controlled viral rebound. Their viral load

was either undetectable or 460 copies/mL and they spontaneously controlled the virus during the 3-week treatment interruption.

9. Three weeks of HAART in combination with DermaVir followed this therapy interruption. All animals controlled virus replication. This treatment period was followed by a three-week therapy interruption period.

10. During the third interruption period all the animals controlled virus replication.

11. The animals exhibited enhanced virus-specific T cell-mediated immune responses after treatment with DermaVir. CD8VIR(SIV) responses of the two animals before DermaVir administration were 1,300 and 4,300 positive cells per 10^6 total CD8 cells and after 3 rounds of DermaVir applications were 32,600 and 6,400 positive cells per 10^6 total CD8 cells, respectively. T cell immunity has been shown to control HIV and SIV replication after interruption of treatment of acute infection. Here we showed that DermaVir induced increased CTL immune responses in monkeys with AIDS and that these immune responses were associated with control of virus replication as well as significantly improved the survival time for the primates. This result, control of virus replication after the interruption of drug treatment during chronic infection or AIDS, is new.

12. These unexpected results showed the antiviral efficacy of DermaVir: DermaVir therapy induced immune control of virus replication during treatment interruptions. Importantly, these data were obtained with chronically infected animals showing signs of AIDS. These results warrant the testing of DermaVir therapy in chronically infected individuals.

13. I HAVE BEEN WARNED THAT WILLFUL FALSE STATEMENTS AND THE LIKE MADE IN THIS DECLARATION ARE PUNISHABLE BY FINE OR IMPRISONMENT, OR BOTH (18 USC 1001) AND MAY JEOPARDIZE THE VALIDITY OF THIS APPLICATION OR ANY PATENT ISSUING THEREON.

______________________ Dr. Julianna Lisziewicz

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